Week 8 Notebook

July 20, 2015 - July 24, 2015

July 20, 2015

Kayla/Julie

- Prepared 1 mL 1:100 dilutions of EMG2:Kλ, 26C, MoAFP, RiAFP, and TiAFP in LB and M9
- Set up new biofilm plate and placed in 37ºC incubator
    - A1-D1 and A6-D6=EMG2:Kλ in LB
    - A2-D2 and A7-D7=26C in LB
    - A3-D3 and A8-D8=MoAFP in LB
    - A4-D4 and A9-D9=RiAFP in LB
    - A5-D5 and A10-D10=TiAFP in LB
    - E1-H1 and E6-H6=EMG2:Kλ in M9
    - E2-H2 and E7-H7=26C in M9
    - E3-H3 and E8-H8=MoAFP in M9
    - E4-H4 and E9-H9=RiAFP in M9
    - E5-H5 and E10-H10=TiAFP in M9
- Streaked 3 plates with 29C5, 30C1, and 31C4 glycerol stocks

Chloe/Charlotte

Colony PCR for the rest of the AFP + 28C or 27C constructs was performed per Dave's protocol.

Liquid cultures of promising colonies from the previous colony PCR were grown up overnight.

Dave/Eddie

- We have confirmed MaAFP (15-33C) and LpAFP (15-35C)!
- We did colony PCR for the same plates from before as well as the new 15-45C plate, using the AFP specific 5' primer and the biobrick 3' primer:
    - 15-38C MpAFP (3)
    - 15-41C DcAFP (2)
    - 15-22C BrAFP (3)
    - 15-32C ChAFP (3)
    - 15-34C EpAFP (3)
    - 15-39C CfAFP (1)
    - 15-42C NgAFP (2)
    - 15-44C HhAFP (3)
    - 15-45C PpAFP (3)
    - Positive Control

    - Negative Control
- We are responsible for making parts pages in the registry, we will begin by giving our parts biobrick numbers.
- Picked colonies 1 and 2 from the 15-20C plate and colony 4 from the 15-23C plate for growth overnight.
- Test digested 15-36C miniprep that was sequenced confirmed and the miniprep made from the picked colony 6.
- Began work on the Parts registration process, using this page:
    - http://parts.igem.org/Help:Adding_Parts

July 21, 2015

Kayla/Julie

- Checked 29C5, 30C1, and 31C4 streak plates
    - No growth on 29C5
        - Prepared 1mL liquid culture from 29C5 glycerol stock to determine if stock was still viable
        - Transformed 29C5 into DH5α to create a new stock
- Miniprepped ZeAFP
    - [DNA]=726 ng/μL
    - Transformed into EMG2:Kλ along with MaAFP(15-33C) and LpAFP(15-35C)

Chloe/Charlotte

The colony PCR products from 7/20 were run on a series of gels. The following numbered colonies were determined to be promising: 35, 36, 57, 60

The cultures grown overnight--Ap28, Ti28, Hh28, Dc28 (1&2), Mp28 (1&2), Ch28--were miniprepped and sent for sequencing. Since the spectrophotometer is malfunctioning, the concentration was unknown, and 5 ul of miniprep and 3 ul of water were submitted as the 8 ul sample for all samples.

Dave's Gel		Gel 1		Gel 2		Gel 3		Gel 4		Control	
1	2	3	4*	5*	6	7*	8*	9*	10*	11*	12*
Ze27	Ze27	Ze27	Mp27	Mp27	Mp27	Ch27	Ch27	Ch27	Ng27	Ng27	Ng27
0.43 kb			1.33 kb			0.8 kb			1.09 kb		
13*	14*	15*	16*	17	18	19	20	21* ?	22	23*	24* ?
Lp27	Lp27	Lp27	Tm27	Tm27	Tm27	Mo27	Mo27	Mo27	Ga27	Ga27	Ga27
0.72 kb			0.71 kb			0.49 kb			0.85 kb		
25*	26* ?	27	28*	29*	30*	31*	32	33*	34	35	36

Ep27	Ep27	Ep27	Br27	Br27	Br27	Cf27	Cf27	Cf27	Ng28	Ng28	Ng28
0.56 kb			0.85			0.68 kb			1.7 kb		
37	38	39*	40*	41*	42*	43*	44*	45*	46*	47*	48*
Ma27	Ma27	Ma27	IAF GP27	IAF GP27	IAF GP27	Ti27	Ti27	Ti27	Ri27	Ri27	Ri27
0.62 kb			0.63 kb			1.09 kb			0.65 kb		
49	50	51	52*	53	54	55	56	57	58	59	60
Ma28	Ma28	Ma28	Hh27	Hh27	Hh27	Tm28	Tm28	Tm28	Mo28	Mo28	Mo28
1.3 kb			1.03 kb			1.4 kb			1.3 kb		
61	62	63	64	65	66	67	68	69			
Lp28	Lp28	Lp28	Cf28	Cf28	Cf28	27C	28C	Ze			
1.6 kb			1.5 kb			150bp	0.8 kb	0.2 kb			

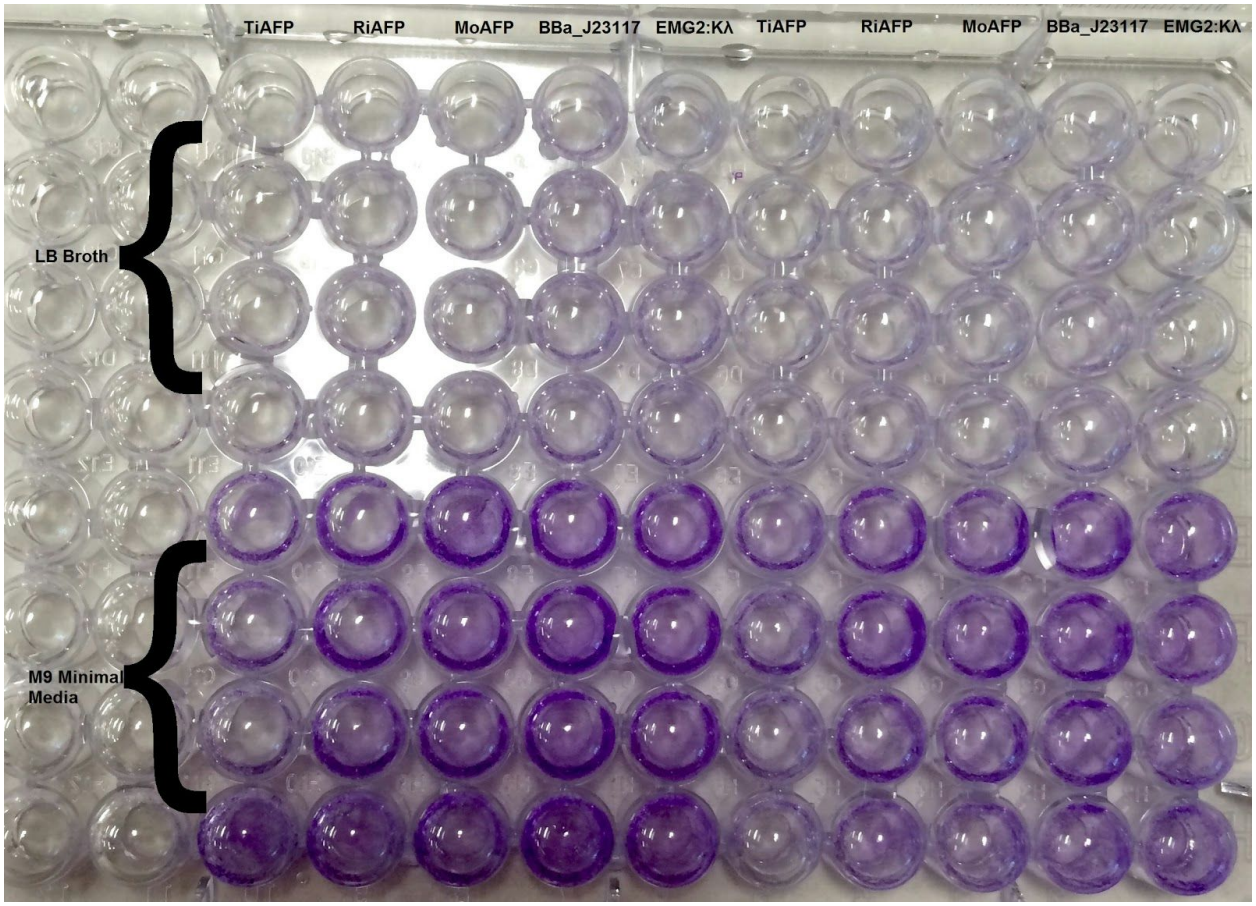
Dave/Eddie

- Made glycerol stocks and miniprepped the two 15-20C cultures and the 15-23C culture and sent them all away for sequencing.
- Ran a gel to examine the test digests from yesterday, with the sequence confirmed miniprep first, the picked colony 6 second, and 3 ul of uncut 15-36C third.
- Ran two gels for the colony PCR, with the wells in order as stated yesterday for the PCR itself, along with ladders at the beginning of each row and a positive and negative control at the end of each gel.
- We realized there was a problem with MoAFP, the sequencing is bad. We are unsure how we failed to recognize this before.
- We are re-ligating MoAFP with CIP treated vector in 2:2 and 2:5 vector to insert ratios
- Picked four colonies of 15-20C to test using J23119-1 and VR for our colony PCR primers, along with a positive control from the glycerol stock of 15-35C and a negative control from the control plate, and ran the colony PCR.

July 22, 2015

Kayla/Julie

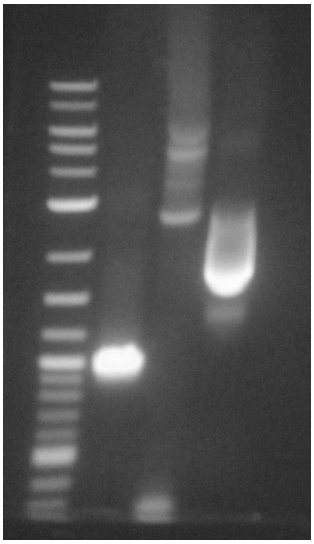
- Transformed GaAFP (15-20C) into EMG2:Kλ
- Stained biofilm plate from 7/20/15 with crystal violet
    - staining shows reduced biofilm formation in EMG2:Kλ expressing TiAFP grown in M9 minimal media



- Prepared 5 mL overnight cultures of EMG2:Kλ, 26C, LpAFP, MaAFP, RiAFP, TiAFP, and ZeAFP

Chloe/Charlotte

The sequences sent out yesterday were analyzed and it was found that while it was thought that all the constructs contained the GFP tag, they actually contained the BclA tag. The gels from last week were analyzed based on this new knowledge. To try and determine what step the samples were mixed up in, small samples from stock tubes of 27C and 28C were sent for sequencing and the digests used in last week's ligation were ran on a gel, pictured below. New digests of 27C and 28C were also prepared.



The analysis of last week's colony PCR (known as colony PCR 1) resulted in the following colony numbers being determined to be promising, and to be grown up in liquid culture overnight: 1, 3, 4, 5, 7, 9, 13, 19, 37

The new freeze survival assay was conducted in 4 different media (LBG, LBG resuspended, PBS, and PBSG) in order to troubleshoot and optimize the assay.

Dave/Eddie

- We got GaAFP, for sure. Totally got it. Radical.
- Column purified the digested, PCR amplified antifreeze proteins, except for GaAFP (15-20C) because we no longer need it.
- CIP treated more vector and column purified.
- Ligated the newly digested AFPs with CIP treated vector, 2 ul of vector and 5 ul of insert.
- Transformed the ligations and plated the transformations.
- Ran a gel to examine the colony PCR.
- Test digested the miniprep of 15-35C to verify that we have the correct miniprep.
- Ran a test digest of LpAFP, see gel. The first well is the miniprep digested with E and P, the second is an undigested control.

July 23, 2015

Kayla/Julie

- Prepared 1:100 dilutions of EMG2:Kλ, 26C, LpAFP, MaAFP, RiAFP, TiAFP, and ZeAFP cultures in LB and M9
    - Measured OD_{600nm} of each dilution to make sure cell counts were similar
    - Plated dilutions
- Completed the interlab study

Chloe/Charlotte

The cultures for the freeze survival assay were taken out of the freezers and the second half of the assay was conducted.

While the assays for -20 freeze and -80 freeze were conducted one at a time, the cultures were thawed at the same time. In the future, the -20 and -80 cultures will be thawed at different times and the assays will be conducted separately in order to improve the timing and efficiency of the assay.

17C MTS Freeze Assay Results (OD_{600}) 7/23					average	avg - blank
LBG Standard Pre-freeze	0.529	0.449	0.427	0.418	0.45575	0.17275
LBG Standard After -20 freeze	0.412	0.528	0.434	0.408	0.4455	0.1245
LBG Standard After -80 freeze	0.361	0.334	0.329	0.389	0.35325	0.02925
LBG Resuspension Pre-freeze	0.529	0.449	0.427	0.418	0.45575	0.17275
LBG Resuspension After -20 freeze	0.387	0.414	0.421	0.436	0.4145	0.0935
LBG Resuspension After -80 freeze	0.338	0.325	0.35	0.348	0.34025	0.01625
PBS Pre-freeze	0.508	0.581	0.577	0.515	0.54525	0.31125
PBS After -20 freeze	0.386	0.397	0.365	0.341	0.37225	0.11825
PBS After -80 freeze	0.288	0.277	0.281	0.283	0.28225	0.02825
PBSG Pre-freeze	0.553	0.701	0.776	0.606	0.659	0.376
PBSG After -20 freeze	0.404	0.445	0.429	0.415	0.42325	0.18225
PBSG After -80 freeze	0.291	0.318	0.297	0.282	0.297	-0.009

19C MTS Freeze Assay Results (OD_{600}) 7/23					average	avg - blank
LBG Standard Pre-freeze	0.538	0.524	0.402	0.434	0.4745	0.1915
LBG Standard After -20 freeze	0.501	0.519	0.519	0.498	0.50925	0.18825
LBG Standard After -80 freeze	0.363	0.382	0.367	0.366	0.3695	0.0455
LBG Resuspension Pre-freeze	0.538	0.524	0.402	0.434	0.4745	0.1915
LBG Resuspension After -20 freeze	0.456	0.423	0.43	0.401	0.4275	0.1065
LBG Resuspension After -80 freeze	0.372	0.357	0.343	0.323	0.34875	0.02475
PBS Pre-freeze	0.42	0.418	0.401	0.387	0.4065	0.1725
PBS After -20 freeze	0.359	0.341	0.349	0.336	0.34625	0.09225

PBS After -80 freeze	0.31	0.311	0.284	0.309	0.3035	0.0495
PBSG Pre-freeze	0.487	0.527	0.532	0.523	0.51725	0.23425
PBSG After -20 freeze	0.442	0.5	0.492	0.487	0.48025	0.23925
PBSG After -80 freeze	0.287	0.313	0.289	0.102	0.24775	-0.05825

21C MTS Freeze Assay Results (OD_{600}) 7/23					average	avg - blank
LBG Standard Pre-freeze	0.506	0.477	0.604	0.535	0.5305	0.2475
LBG Standard After -20 freeze	0.502	0.539	0.515	0.289	0.46125	0.14025
LBG Standard After -80 freeze	0.38	0.43	0.356	0.366	0.383	0.059
LBG Resuspension Pre-freeze	0.506	0.477	0.604	0.535	0.5305	0.2475
LBG Resuspension After -20 freeze	0.533	0.495	0.493	0.515	0.509	0.188
LBG Resuspension After -80 freeze	0.441	0.423	0.361	0.364	0.39725	0.07325
PBS Pre-freeze	0.33	0.331	0.338	0.337	0.334	0.1
PBS After -20 freeze	0.272	0.29	0.285	0.272	0.27975	0.02575
PBS After -80 freeze	0.296	0.317	0.333	0.288	0.3085	0.0545
PBSG Pre-freeze	0.41	0.42	0.425	0.446	0.42525	0.14225
PBSG After -20 freeze	0.457	0.419	0.391	0.398	0.41625	0.17525
PBSG After -80 freeze	0.344	0.341	0.31	0.303	0.3245	0.0185

Dave/Eddie

- Ran colony PCR on the same plates using J23119-1 and VR:
    - 15-38C (5)
    - 15-41C (4)
    - 15-45C (5)
    - 15-22C (5)
    - 15-32C (3)
    - 15-34C (5)
    - 15-39C (3)
    - 15-42C (4)
    - 15-44C (5)
    - Positive Control
    - Negative Control
- Three colonies looked good and were picked for liquid culture growth overnight:
    - 15-22C #3

    - 15-34C #5
    - 15-42C #4
- Miniprepped the liquid culture of 15-35C, LpAFP, that was grown up from the glycerol stock. A test digest was performed. We realized we don't have LpAFP and that the sequencing doesn't actually look good.
- PCR amplified CsAFP with 10 ul of master mix, 9 ul of DNA, and 0.5 ul of each of the biobrick primers. Digested 5 ul of the amplified AFP with X and P.

July 24, 2015

Chloe/Charlotte
A 50 ul digest of 28C was prepared using S and P, and a 20 ul test digest of 28C was prepared using E and P. They were incubated at 37 C for two hours.

A new set of ligations and transformations of GFP+AFPs was started, but the gel run of the new test digest of 28C showed that there was no vector present, as there was only one band on the gel representing GFP and nothing present for the rest of the plasmid. Because of this, the new set of transformations was tossed.

Dave/Eddie
- Digested LpAFP insert with X and P.
- Made glycerol stocks and miniprepped 15-22C, 15-34C, and 15-42C, and sent away samples for sequencing.
- Ran a colony PCR for the plates made on 7/22/15:
- Ran the colony pcr on three gels, the last two wells were positive and negative controls respectively. The following order:
    - 38C [5]
    - 41C [5]
    - 45C [5]
    - 23C [4]
    - 32C [4]
    - 36C [5]
    - 37C [5]
    - 39C [5]
    - 40C [5]
    - 44C [5]
    - Due to accidentally skipping the tenth well in the first row, the first gel is ladder, 1-8,15, 9-14,16, + then - controls.
- Ligated 15-35C and 15-46C twice each, once with 2 ul of insert and once with 5 ul of insert. Transformed all four and a control and left to grow overnight.

July 25, 2015

Kayla/Julie

- Stained biofilms from the 7/23 biofilm plate

Dave/Eddie

- Moved the plates to the fridge.
- Spun down the liquid cultures and decanted the liquid, leaving them in the freezer for the rest of the weekend.

July 26, 2015

Kayla/Julie

- Stained biofilms from the 7/24 biofilm plate
    - some biofilm formation in the LB wells due to media contamination